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Comparison of selected wheat, oat and buckwheat genotypes on proteomic level

Porovnanie vybraných genotypov pšenice, ovsa a pohánky na proteomickej úrovni

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ABSTRACT

Cereals and pseudocereals are one of the most important crops. In recent years, number of people suffering from various food allergies and intolerances has grown and exactly, cereal proteins are the most common food allergen group. Pseudocereals are suitable alternative that have positive effect on human organism and do not induce allergies. The aim of this work was to analyze grains of winter wheat (*Triticum aestivum* L.), oat (*Avena sativa*) and buckwheat (*Fagopyrum esculentum* Moench.) and compare protein composition from the point of nutritional and technological quality. Wheat had the best technological quality based on the highest content of gluten proteins (63.42%) and HMW-GS (15.21%). Potentially allergenic wheat proteins with molecular weight in the range of 25 to 45 kDa gave strong signal in Western blot. Oat, due to the higher coefficient of nutritional quality (373.89%) and content of albumins and globulins (49.29%), had worse technological and better nutritional quality than wheat. The best nutritional quality was based on the highest coefficient of nutritional quality (6,005.93%), content of albumins and globulins (60.81%) and essential amino acids in buckwheat. Buckwheat is suitable crop for gluten-free diet because it contained only 1.35% of prolamins that were not detected by Western blot analysis.

Keywords: buckwheat, oat, proteins, wheat

ABSTRAKT

Obilniny a pseudoobilniny sú jednými z najdôležitejších poľnohospodárskych plodín. V posledných rokoch narastá počet ľudí trpiacich rôznymi potravinovými alergiami a intoleranciami a práve bielkoviny obilnín predstavujú najčastejšiu skupinu potravinových alergénov. Vhodnou alternatívou sú pseudoobilniny, ktoré majú pozitívny vplyv na ľudský organizmus a nevyvolávajú alergie. Cieľom tejto práce bolo analyzovať zrná pšenice letnej (*Triticum aestivum* L.), ovsa siateho (*Avena sativa*) a pohánky siatej (*Fagopyrum esculentum* Moench.) a porovnať bielkovinové zloženie z pohľadu nutričnej a technologickej kvality. Pšenica vykazovala na základe najvyššieho obsahu gluténových bielkovín (63,42%) a HMW-GS (15,21%) najlepšiu technologickú kvalitu. Potenciálne alergénne pšeničné bielkoviny s molekulovou hmotnosťou 25 až 45 kDa dávali vo Western blot silný signál. Ovos mal vzhľadom na vyšší koeficient nutričnej kvality (373,89%) a obsah albumínov a globulínov (49,29%) horšiu technologickú a lepšiu nutričnú kvalitu ako pšenica. Najlepšiu nutričnú kvalitu sme na základe najvyššieho koeficienta nutričnej kvality (6 005,93%), obsahu albumínov a globulínov (60,81%) a esenciálnych aminokyselín stanovili v pohánke. Pohánka je vhodnou plodinou v bezlepkovej diéte pretože obsahovala len 1,35% prolamínov, ktoré neboli detekované Western blot analýzou.

Kľúčové slová: bielkoviny, ovos, pohánka, pšenica

INTRODUCTION

Cereals are a globally consumed crop and have an important role in human nutrition. They are source of proteins, energy, minerals, vitamins, dietary fibre and phytochemicals (Belitz et al., 2009).

Wheat (*Triticum aestivum* L.) is one of the most cultivated cereal crops in the world. Proteins of the wheat flour can be divided into albumins, globulins, gliadins and glutenins according to their solubility in water, salt solution, aqueous ethanol or dilute acid or alkali. Wheat proteins, on the basis of their functional properties, can be divided into gluten (storage) proteins and non-gluten (cytoplasmatic) proteins. According to that, wheat is especially used for bread-making because grains contains high amount of gluten proteins. However, this protein fraction is unsuitable for people who have intolerance to gluten proteins in cereals and suffer from a celiac disease (Osborne, 1907; Lagarón, 2011; Hager et al., 2012; Wang et al., 2016).

Oat belongs to the grass family Poaceae as wheat. It contains relatively high amount of proteins and oat proteins have excellent nutritional score of amino acids. The major storage proteins of oats are globulins. Oat has a higher content of glutelins than wheat and the prolamins composition of oat is intermediate between Triticeae and the other cereals (Belitz et al., 2009; Gregová et al., 2015).

Buckwheat is dicotyledonous plant as opposed to most cereals, which are monocotyledonous. It is not true cereal but is referred to as pseudocereal because grains of buckwheat are resembled in composition and function to cereal grains. Buckwheat is a good source of nutritionally important elements. Proteins of buckwheat contain little amount of prolamins, which are the main cereal storage proteins. On the other side, buckwheat proteins are composed mainly of albumins and globulins (Alvarez-Jubete et al., 2010).

In recent years, great emphasis has been put on the production of foods that are suitable for gluten-free diet (Duta and Culetu, 2015). Food allergens include proteins or glycoproteins with a molecular weight of 5 to 100 kDa and ability to bond IgE receptors (Breiteneder and Radauer, 2004). Reason of problems about patients with coeliac disease are prolamine fractions of cereal gluten with low molecular weight around 30 kDa (Belitz et al., 2009). The major pseudocereal storage proteins are similar to leguminous proteins. They do not contain gluten, and therefore are a good food for people suffering from celiac disease (Taylor et al., 2016). Method used to searching for food allergens is Western blot which employes immunochemical detection by specific antibodies (Hench and Jones, 2005).

MATERIALS AND METHODS

Plant material

For the analyses, grains of winter wheat (*Triticum aestivum* L.) genotype PS Puqa, common oat (*Avena sativa*) genotype Zvolen and buckwheat (*Fagopyrum esculentum* Moench.) genotype Špačinská 1 from Slovakia were obtained from Research and Breeding Station at Vígľaš-Pstruša – The Research Institute of Plant Production, Piešťany. Analyzed genotypes represent average characteristics of selected species.

Total nitrogen content

In all analyzed samples, total nitrogen content was determinated in 5 biological replicates by Kjeldahl method (1983). In presence of $CuSO_4$ and K_2SO_4 as a catalyst with concentrated H_2SO_4, 0.5 g of ground grain was mineralized for 20 minutes at 420 °C. After distillation, titration of ammonia was done by using of Tashiro's indicator with 0.05 mol/dm^3 H_2SO_4.

Fractional composition of proteins

All protein fractions from analyzed samples were extracted according to Michalík (2002) and the content of protein was measured and calculated following Kjeldahl in 5 biological replicates for each sample.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis

Storage proteins of the grain were isolated from whole, dry, mature grains and were extracted from individual grains according to standard method by ISTA (Wrigley, 1992). Separation of storage proteins was performed in polyacrylamide gel in the presence of sodium dodecyl sulfate in the vertical discontinual electrophoretic system Hoefer SE 600 DeLuxe by Thermo Fisher Scientific by the standard reference electrophoretic method by ISTA (Wrigley, 1992). Gels were coloured in Comassie Brilliant Blue R250. Electrophoretic profiles were visualized in photo device with black and white CCD camera and then were analyzed in Doc-It LS Image analysis UVP software.

Western blot

Proteins for Tricine-SDS-PAGE according to Schägger (2006) were extracted in sample buffer with β-mercaptoethanol at 23 °C for 1 hour. After electrophoresis, proteins were electrotransfered to polyvinylidene fluoride membrane Immobilon-P (Millipore) in transfer buffer at 170 mA for 1.5 hour. Antibodies were diluted and membrane was incubated with primary antibody (Anti-gliadin antibody produced in rabbit) and secondary antibody (Goat anti-rabbit IgG) for 1 hour. After each incubation, membrane was washed with buffer 5 times for 10 minutes. Blots were developed by diaminobenzidine tablets dissolved in distilled water (Sigma Aldrich).

RESULTS AND DISCUSSION

One of the most important sources of food proteins are cereals. In analyzed genotypes of wheat, oat and buckwheat were determined crude protein content and Coefficient of nutritional quality. Measurements were performed in 5 repetitions. Crude protein content (Table 1) in buckwheat was 12.43%, while the lowest value was detected in wheat (10.21%). The highest value of coefficient of nutritional quality (Table 1), as a indicator of nutritional quality, was reached in buckwheat (6,005.93) and the lowest in wheat (115%). Coefficient of nutritional quality in oat was 373.89%. In another study of Rajnincová et al. (2018), average crude protein content in wheat was 9.9%. Gálová et al. (2011) analyzed genotypes of different crops and crude protein content was 8% to 11.35% in wheat, 9.81% to 12.19% in oat and 9.51 to 10.35% in buckwheat which was consistent with this results.

Table 1. Average nitrogen and protein content in analyzed samples

	PS Puqa[1]	Zvolen[2]	Špačinská 1[3]
Total nitrogen (%)	1.79	1.96	2.07
Crude protein content (%)	10.21	11.43	12.43
Coefficient of nutritional quality (%)	115	373.89	6,005.93

[1] PS Puqa – genotype of winter wheat *Triticum aestivum* L., [2] Zvolen – genotype of common oat *Avena sativa* L., [3] Špačinská 1 – genotype of buckwheat *Fagopyrum esculentum* Moench.

The basic function of proteins in nutrition is to provide adequate amounts of suitable amino acids. Protein quality depends mainly on content of amino acid and essential amino acid. The metabolism of proteins is determined by the ratio of amino acids used for protein synthesis (Matuz et al., 2000).

The availability of amino acids varies depending on the source of the protein, the interaction with other dietary components, and processing in the food industry (Matuz et al., 2000). The buckwheat proteins have a high biological value in regard to the balanced amino acid composition, although their digestibility is relatively low. Compared with wheat flour proteins, buckwheat proteins have a higher or similar content of almost all amino acids except glutamine and proline, whose percentage is much lower than in wheat flour (Krkošková and Mrázová, 2005). The main oat protein is globulin, which is more rich in essential amino acids than the prolamine proteins of the botanical group Triticeae (Mäkinen et al., 2017).

Content of some amino acids was specified in cooperation with Research Institute for Animal Production in Nitra. The highest total content of amino acids (Table 2) was determined in buckwheat (131.72 mg/g dry weight) compared with wheat (115.76 mg/g dry weight) and oat

Table 2. Average amino acid composition of analyzed proteins

Amino acid (mg/g dry weight)	PS Puqa[1]	Zvolen[2]	Špačinská 1[3]
Aspartic acid	7.94	14.3	15.52
Threonine	4.25	5.51	6.27
Serine	5.62	6.35	7.68
Glutamic acid	38.89	29.18	27.52
Proline	11.43	6.16	5.53
Glycine	5.04	6.22	8.81
Alanine	3.57	5.71	5.83
Valine	3.67	5.59	5.32
Isoleucine	2.95	4.1	4
Leucine	7.72	9.27	8.54
Tyrosine	2.9	3.51	2.96
Phenylalanine	4.86	5.93	5.53
Histidine	3.35	3.37	4
Lysine	3.13	4.7	7.54
Arginine	5.35	7.74	11.86
Cystine	3	2.48	3.28
Methionine	2.1	1.46	1.54
Sum total	115.76	121.57	131.72

[1] PS Puqa – genotype of winter wheat *Triticum aestivum* L., [2] Zvolen – genotype of common oat *Avena sativa* L., [3] Špačinská 1 – genotype of buckwheat *Fagopyrum esculentum* Moench

(121.57 mg/g dry weight). One of the most abundant amino acid in wheat was non-essential proline (11.43 mg/g dry weight) and aspartic acid in oat (14.3 mg/g dry weight) and buckwheat (15.52 mg/g dry weight). Oat and buckwheat had higher content of essential amino acids than wheat. The most abundant essential amino acid in all crops was leucine (7.72 mg/g dry weight wheat – 9.27 mg/g dry weight oat) and the lowest was methionine (1.46 mg/g dry weight oat– 2.1 mg/g dry weight wheat). The lysine content, as the limiting amino acid, was the highest in buckwheat (7.54 mg/g dry weight) compared with wheat (3.13 mg/g dry weight) and oat (4.7 mg/g dry weight). This results were comparable with Arendt and Zannini (2013).

According to nutritional and technological quality, the fractional composition of the protein complex of the grain has major importance because it specifies utilization of the grain for food or feed purposes. The content of albumins and globulins influences especially nutrition quality, on the other hand the amount of prolamins and glutenins affects the technological quality of the grain. (Palenčárová and Gálová, 2009; Chňapek et al., 2010).

In the analyzed samples, fractional composition of proteins was performed in 5 repetitions (Table 3). The highest content of albumins and globulins was determined in buckwheat (60.81%) but content of prolamins and glutelins was the lowest right in this pseudocereal (18.92%). On the other side, the highest content of prolamins and glutelins (63.42%) was detected in wheat, which had the lowest content (28.84%) of albumins and globulins. Content of albumins, globulins (49.29%) and prolamins, glutelins (38.57%) of oat vary between content of wheat and buckwheat.

Table 3. Protein fractional composition of analyzed samples

	PS Puqa[1]	Zvolen[2]	Špačinská 1[3]
Albumins+globulins (%)	28.84	49.29	60.81
Prolamins+glutelins (%)	63.42	38.57	18.92

[1] PS Puqa – genotype of winter wheat *Triticum aestivum* L., [2] Zvolen – genotype of common oat *Avena sativa* L., [3] Špačinská 1 – genotype of buckwheat *Fagopyrum esculentum* Moench.

Prolamins are the main cause of problems in people suffering from coeliac disease. Pseudocereals had low content of gluten therefore they are suitable food for people suffering from celiac disease. In Figure 1, buckwheat contains almost none prolamins (1.35%).

The highest content of prolamines was detected in wheat 31.81% and oat had 16.43% of prolamines. Pešková et al. (2017) determined, the average content of albumins and globulins in analyzed wheat was 24.55% and prolamins and glutelins 65.98%. According to Socha (2011), content of albumins and globulins in buckwheat was 48.6% and prolamins and glutelins 19.63%, that corresponded with this results.

The most widespread method used to identify high molecular weight glutenin subunits is polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE). The electrophoretic spectrum of the proteins of analyzed crops in SDS-PAGE is represented by Figure 2. According to molecular weight, proteins were separated into high molecular weight glutenin subunits (HMW-GS) upside, low molecular weight glutenin subunits (LMW-GS) in the middle part of the polyacrylamide gel and the residual albumins and globulins.

The widest protein profile was identified in buckwheat, molecular weight was in the range of 210 kDa to 3 kDa (Table 4). Molecular weight of wheat proteins was from 140 kDa to 2 kDa and oat was in the range of 97 kDa to 2 kDa. The most intensive bands with molecular weight around 30 kDa, which are potentially allergenic, were found in wheat (Figure 2).

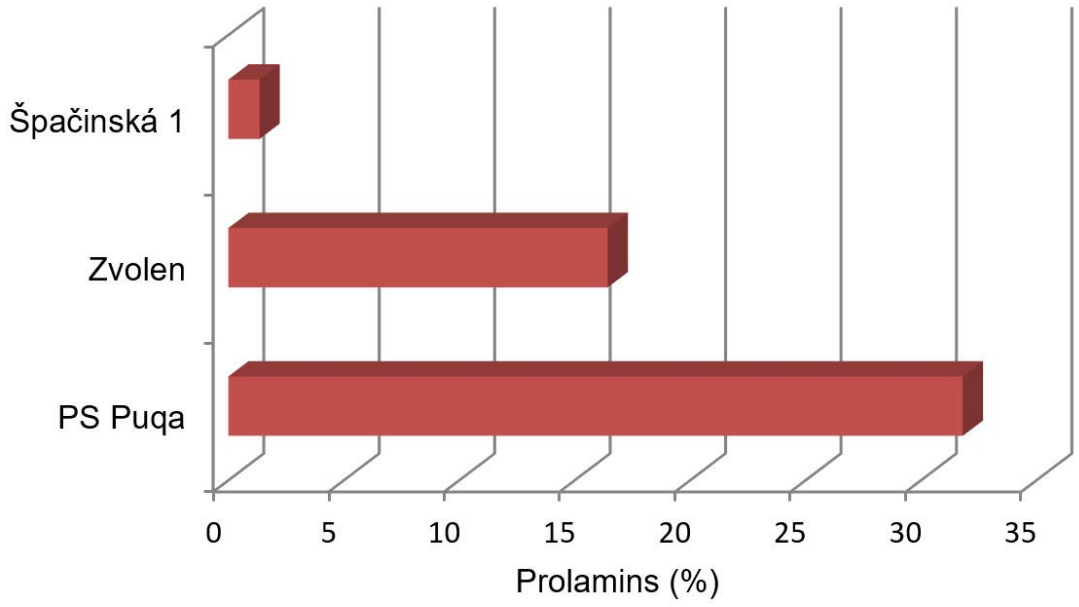

Figure 1. Content of prolamines (PS Puqa – genotype of winter wheat *Triticum aestivum* L., Zvolen – genotype of common oat *Avena sativa* L., Špačinská 1 – genotype of buckwheat *Fagopyrum esculentum* Moench.)

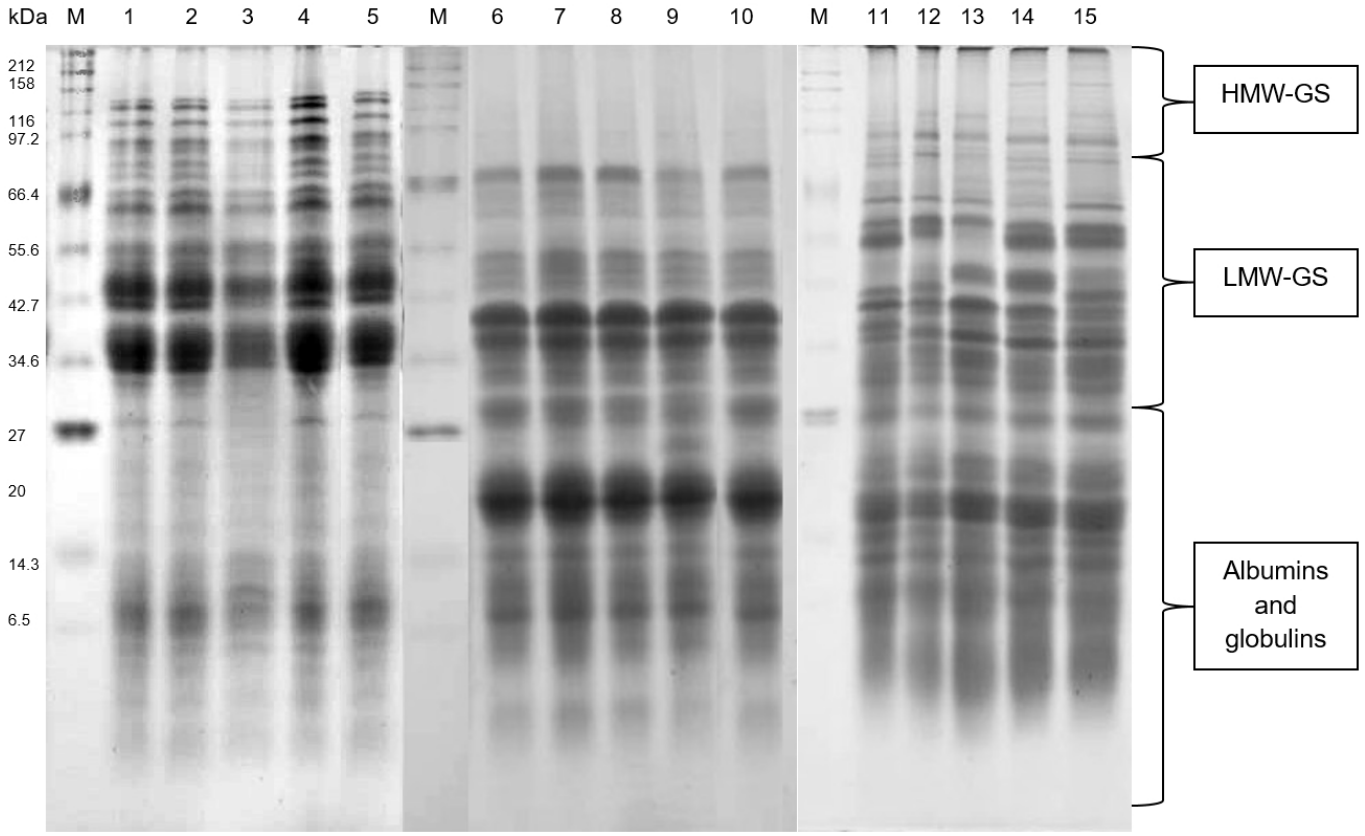

Figure 2. Electrophoretic spectrum of proteins in SDS-PAGE (HMW-GS – high molecular weight glutenin subunits, LMW-GS – low molecular weight glutenin subunits, M – molecular marker P7702S, 1-5 – *Triticum aestivum* L. genotype PS Puqa, 6-10 – *Avena sativa* L. genotype Zvolen, 11-15 – *Fagopyrum esculentum* Moench. genotype Špačinská 1.)

Table 4. Molecular weight of analyzed proteins

Fractions of storage proteins	Molecular weight of protein subunits (kDa)		
	PS Puqa	Zvolen	Špačinská 1
HMW-GS[1]	140-95	97-95	210-95
LMW-GS[2]	90-30	70-30	90-30
Alb+glo[3]	27-2	30-2	27-3

[1] HMW-GS – high molecular weight glutenin subunits, [2] LMW-GS – low molecular weight glutenin subunits, [3] alb+glo – albumins and globulins.

Electrophoreograms of all genotypes were analyzed and differences in glutenin subunits were found. Percentage of HMW-GS, LMW-GS, and residual albumins and globulins were determined based on the separation of storage proteins of analyzed crops in SDS-PAGE (Table 5).

In the analyzed samples of wheat (Table 5), the highest percentage (64.58%) had LMW-GS and the lowest amount represent HMW-GS (15.21%).

Results showed, that oat and buckwheat had different percentage of proteins, which were separated in SDS-PAGE, compared to wheat (Table 5). The higher content of proteins in oat and buckwheat reached LMW-GS (oat 33%; buckwheat 39%) and the lower percentage was

Table 5. Percentage of HMW-GS and LMW-GS in analyzed wheat, oat and buckwheat

Genotype	HMW-GS[1] (%)	LMW-GS[2] (%)	Alb+glo[3] (%)
PS Puqa[4]	15.21 ± 0.73	64.58 ± 1.3	20.21 ± 1.67
Zvolen[5]	6.75 ± 0.61	33.44 ± 2.44	59.12 ± 2.26
Špačinská 1[6]	10.39 ± 0.95	39.01 ± 1.05	50.61 ± 1.16

[1] HMW-GS (%) - high molecular weight glutenin subunits; [2] LMW-GS (%) – low molecular weight glutenin subunits; [3] alb+glo (%) – albumins and globulins; [4] PS Puqa – genotype of winter wheat *Triticum aestivum* L., [5] Zvolen – genotype of common oat *Avena sativa* L., [6] Špačinská 1 – genotype of buckwheat *Fagopyrum esculentum* Moench. Values represent mean ± standard deviation.

detected in HMW-GS (oat 6.8%; buckwheat 10.4%). The highest percentage of HMW-GS and LMW-GS was detected in wheat and the lowest in oat. Buckwheat, such as pseudocereal, had content of HMW-GS and LMW-GS only a few percent higher than oat (Figure 3).

This results were comparable to the results of Gálová et al. (2011), which determined the HMW-GS content from 2.6% to 28.41% in wheat, LMW-GS from 54.5% to 83.88%. HMW-GS content in oats ranged from 0.79% to 2.56%, LMW-GS from 4.62% to 49.06%. In buckwheat, HMW-GS ranged from 0% to 15.57%, LMW-GS from 5% to 54.4%.

Immunological features were investigated by reaction of prolamins with polyclonal anti-gliadin antibody by Western blot. According to the literature, the most coeliac active proteins are prolamins with molecular weight around 30 kDa. In sample of wheat, polyclonal antibody recognized all protein fractions of molecular weight higher than 25 kDa. Antibody did not react with low molecular weight proteins about 13-20 kDa. Wheat proteins with molecular weight in the range of 25 to 45 kDa gave very strong signal and these proteins can be potential coeliac allergens (Figure 4). In oat, antibody react with proteins about 15-50 kDa and did not recognize proteins with molecular weight higher than 55 kDa and lower than 13 kDa. Protein with molecular weight about 20 kDa gave stronger signal than others. In sample of buckwheat, immunoreactive proteins were not detected by Western blot performed with polyclonal anti-gliadin antibody, antibody did not react with any proteins, that mean, prolamins were not recognized in this sample. Similar reactions between polyclonal anti-gliadin antibody and proteins of wheat and oat was presented in study Socha et al. (2011) and Mickowska et al. (2012).

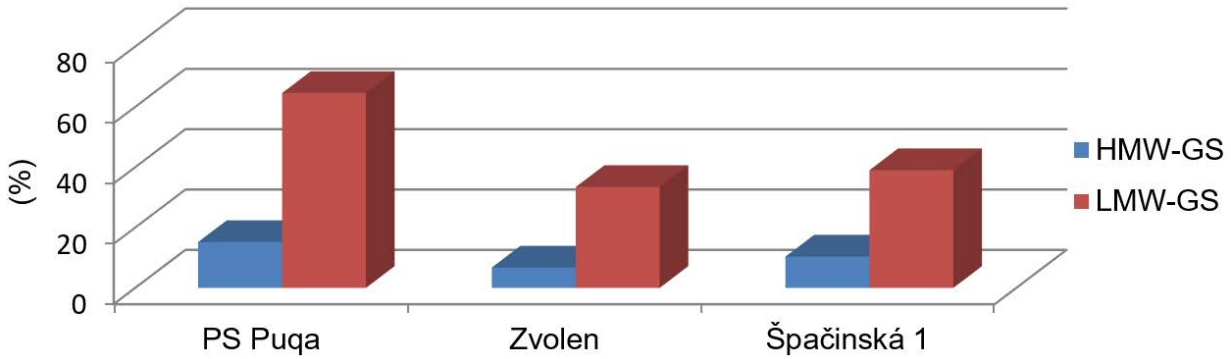

Figure 3. Percentage of HMW-GS, LMW-GS and residual albumins and globulins of wheat, oat and buckwheat (HMW-GS – high molecular weight glutenin subunits, LMW-GS – low molecular weight glutenin subunits)

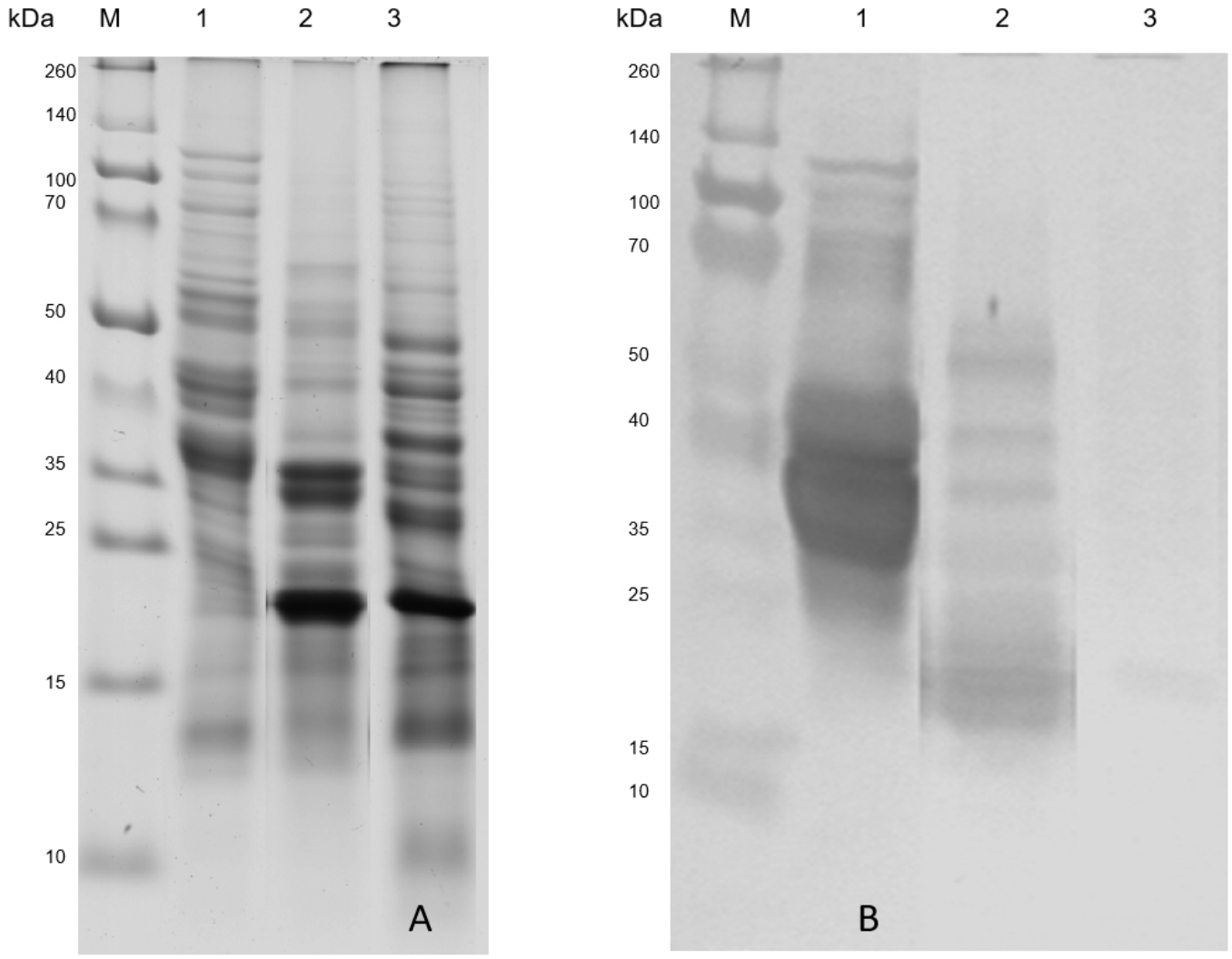

Figure 4. Immunoreactivity of wheat, oat and buckwheat prolamins (A – polyacrylamide gel electrophoresis of analyzed proteins in presence sodium dodecyl sulfate, B – Western blot of analyzed proteins, M – molecular marker Thermo Scientific Spectra Multicolor Broad Range Protein Ladder, 1 – *Triticum aestivum* L. genotype PS Puqa, 2 – *Avena sativa* L. genotype Zvolen, 3 – *Fagopyrum esculentum* Moench. genotype Špačinská 1.)

CONCLUSIONS

Cereals and pseudocereals are the main source of food proteins. The highest crude protein content of the analyzed crops was detected in the buckwheat (12.43%). This genotype showed the best nutritional quality in regard to the highest coefficient of nutritional quality (6,005.93%) and the highest content of albumins and globulins (60.81%) rich in essential amino acids. Buckwheat had the highest content of total amino acids (131.72 mg/g dry weight) and together with oat had a higher content of essential amino acids in comparison to wheat. Wheat showed the highest prolamine and gluteline content (63.42%) and HMW-GS (15.21%) which are indicators of technological quality. The highest content of prolamins, that cause problems to people suffering from celiac disease, was measured in wheat (31.81%). Potentially allergenic wheat proteins with molecular weight in the range of 25 to 45 kDa gave very strong signal in Western blot. Buckwheat contained only 1.35% of prolamins. Immunoreactive proteins was not detected by immunological analysis. Because of these obserations, buckwheat can be considered as suitable food in a gluten-free diet. Oat had a better nutritional and worse technological quality than wheat, considering the

coefficient of nutritional quality (373.89%) and the higher content of albumins and globulins (49.29%). Oat proteins with molecular weight from 15 to 50 kDa were labeled by immunological reaction in Western blot.

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